Hypothalamic thyrotropin-releasing hormone mRNA responses to hypothyroxinemia induced by sleep deprivation

CAROL A. EVERSON1 AND THADDEUS S. NOWAK, JR.2
1Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226; and 2Department of Neurology, University of Tennessee Health Sciences Center, Memphis, Tennessee 38163

Received 19 December 2001; accepted in final form 8 March 2002

Everson, Carol A. and Thaddeus S. Nowak, Jr. Hypothalamic thyrotropin-releasing hormone mRNA responses to hypothyroxinemia induced by sleep deprivation. Am J Physiol Endocrinol Metab 283: E85–E93, 2002.—Sleep deprivation in rats results in progressive declines in circulating concentrations of both total and free thyroxine (T4) and triiodothyronine (T3) without an expected increase in plasma thyroid-stimulating hormone (TSH). Administration of thyrotropin-releasing hormone (TRH) results in appropriate increases in plasma TSH, free T4, and free T3 across experimental days, suggesting deficient endogenous TRH production and/or release. This study examined transcriptional responses related to TRH regulation following sleep deprivation. In situ hybridization was used to detect and quantitate expression of mRNAs encoding prepro-TRH and deiodinase type II (5'-deiodinase, type II DII) in brain sections of six rats sleep deprived for 16–21 days, when there was marked hypothyroxinemia, and in sections from animals yoked to the experimental protocol as well as from sham controls. TRH transcript levels in the paraventricular nucleus (PVN) were essentially unchanged at 15–16 days but increased to about threefold control levels in three of four rats sleep deprived for 20–21 days, a change comparable to that typically found in prolonged experimental hypothyroidism. There was no evidence for suppression of 5'-DII mRNA levels, which would be a sign of T3 feedback downregulation of neurons in the PVN. A failure to increase serum TSH in response to hypothyroxinemia and to increased prepro-TRH mRNA expression indicates that alterations in posttranscriptional stages of TRH synthesis, processing, or release likely mediate the central hypothyroidism induced by sleep deprivation.

T4 and T3 in sleep-deprived rats are considered grossly abnormal, not only because of their progressiveness and severity but also because of the apparent lack of pituitary activation. The free forms of T4 and T3 decline in parallel with the total concentrations, indicating that changes in binding characteristics are not responsible for low T4 (23). Plasma reverse T3 concentrations are not increased into a range that would indicate T4 inactivation (23). T4 shows a greater decline than does T3, to 75% below basal concentrations by the onset of advanced morbidity, compared with a 55% decline in T3 (8). The increased T3-to-T4 ratio likely involves local T3 production by brown adipose tissue (BAT), a major T4-to-T3 conversion pathway in the rat, which can contribute 40% or more of circulating T3 (54). Indeed, the T4-to-T3 conversion enzyme, type II 5 '-deiodinase, in BAT is increased 100-fold in sleep-deprived rats (6), a level consistent with experimental hypothyroidism (13, 54).

Low plasma T4 is a potent stimulator of thyroid-stimulating hormone (TSH) secretion from the pituitary (61). A previous study in sleep-deprived rats indicated that the plasma TSH concentration remains essentially unchanged at 15–16 days but increased to about threefold control levels in three of four rats sleep deprived for 20–21 days, a change comparable to that typically found in prolonged experimental hypothyroidism. There was no evidence for suppression of 5'-DII mRNA levels, which would be a sign of T3 feedback downregulation of neurons in the PVN. A failure to increase serum TSH in response to hypothyroxinemia and to increased prepro-TRH mRNA expression indicates that alterations in posttranscriptional stages of TRH synthesis, processing, or release likely mediate the central hypothyroidism induced by sleep deprivation.

5'-deiodinase type II (5'-DII) in brain sections of six rats sleep deprived for 16–21 days, when there was marked hypothyroxinemia, and in sections from animals yoked to the experimental protocol as well as from sham controls. TRH transcript levels in the paraventricular nucleus (PVN) were essentially unchanged at 15–16 days but increased to about threefold control levels in three of four rats sleep deprived for 20–21 days, a change comparable to that typically found in prolonged experimental hypothyroidism. There was no evidence for suppression of 5'-DII mRNA levels, which would be a sign of T3 feedback downregulation of neurons in the PVN. A failure to increase serum TSH in response to hypothyroxinemia and to increased prepro-TRH mRNA expression indicates that alterations in posttranscriptional stages of TRH synthesis, processing, or release likely mediate the central hypothyroidism induced by sleep deprivation.

T4 and T3 in sleep-deprived rats are considered grossly abnormal, not only because of their progressiveness and severity but also because of the apparent lack of pituitary activation. The free forms of T4 and T3 decline in parallel with the total concentrations, indicating that changes in binding characteristics are not responsible for low T4 (23). Plasma reverse T3 concentrations are not increased into a range that would indicate T4 inactivation (23). T4 shows a greater decline than does T3, to 75% below basal concentrations by the onset of advanced morbidity, compared with a 55% decline in T3 (8). The increased T3-to-T4 ratio likely involves local T3 production by brown adipose tissue (BAT), a major T4-to-T3 conversion pathway in the rat, which can contribute 40% or more of circulating T3 (54). Indeed, the T4-to-T3 conversion enzyme, type II 5'-deiodinase, in BAT is increased 100-fold in sleep-deprived rats (6), a level consistent with experimental hypothyroidism (13, 54).

Low plasma T4 is a potent stimulator of thyroid-stimulating hormone (TSH) secretion from the pituitary (61). A previous study in sleep-deprived rats indicated that the plasma TSH concentration remains essentially unchanged at 15–16 days but increased to about threefold control levels in three of four rats sleep deprived for 20–21 days, a change comparable to that typically found in prolonged experimental hypothyroidism. There was no evidence for suppression of 5'-DII mRNA levels, which would be a sign of T3 feedback downregulation of neurons in the PVN. A failure to increase serum TSH in response to hypothyroxinemia and to increased prepro-TRH mRNA expression indicates that alterations in posttranscriptional stages of TRH synthesis, processing, or release likely mediate the central hypothyroidism induced by sleep deprivation.

There is near universal concurrence that profound and chronic sleep deprivation is a risk factor for illness (1, 2) and that it impairs recovery (5, 14). Although specific physiological changes that may contribute to pathology in the clinical population have yet to be extensively investigated, characteristic findings have emerged from studies in laboratory animals. A typical 3-wk survival period in sleep-deprived rats is marked by hypercatabolism, manifested by increased food intake and loss of body weight without diabetes or malabsorption of calories (8, 22, 27). Body temperature initially increases slightly, by <0.5°C (8). Despite this resemblance to hyperthyroidism, plasma thyroxine (T4) and triiodothyronine (T3) instead decline progressively to very low concentrations (8, 23). Plasma noradrenaline increases (45), whereas plasma corticosteroids remain unchanged (23). Resistance to infectious disease is decreased, revealed by early and continual infection of the mesenteric lymph nodes by bacteria translocated from the intestine, and periodic transient infections of major organs (26). The endocrine changes and the septic state eventuate in an acute condition of advanced morbidity and hypothermia that precede death. Terminal signs and positive blood cultures are consistent with lethal septicemia (21).

The declines in both plasma T4 and T3 in sleep-deprived rats are considered grossly abnormal, not only because of their progressiveness and severity but also because of the apparent lack of pituitary activation. The free forms of T4 and T3 decline in parallel with the total concentrations, indicating that changes in binding characteristics are not responsible for low T4 (23). Plasma reverse T3 concentrations are not increased into a range that would indicate T4 inactivation (23). T4 shows a greater decline than does T3, to 75% below basal concentrations by the onset of advanced morbidity, compared with a 55% decline in T3 (8). The increased T3-to-T4 ratio likely involves local T3 production by brown adipose tissue (BAT), a major T4-to-T3 conversion pathway in the rat, which can contribute 40% or more of circulating T3 (54). Indeed, the T4-to-T3 conversion enzyme, type II 5'-deiodinase, in BAT is increased 100-fold in sleep-deprived rats (6), a level consistent with experimental hypothyroidism (13, 54).

Low plasma T4 is a potent stimulator of thyroid-stimulating hormone (TSH) secretion from the pituitary (61). A previous study in sleep-deprived rats indicated that the plasma TSH concentration remains essentially unchanged at 15–16 days but increased to about threefold control levels in three of four rats sleep deprived for 20–21 days, a change comparable to that typically found in prolonged experimental hypothyroidism. There was no evidence for suppression of 5'-DII mRNA levels, which would be a sign of T3 feedback downregulation of neurons in the PVN. A failure to increase serum TSH in response to hypothyroxinemia and to increased prepro-TRH mRNA expression indicates that alterations in posttranscriptional stages of TRH synthesis, processing, or release likely mediate the central hypothyroidism induced by sleep deprivation.

Central hypothyroidism; preprothyrotropin-releasing hormone messenger ribonucleic acid; paraventricular nucleus; thyroid hormones

Address for reprint requests and other correspondence: C. A. Everson, MCW Neurology Research 151, VA Medical Center, Bldg. 70, 5000 West National Ave., Milwaukee, WI 53295 (E-mail: ceverson@mcw.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpendo.org 0193-1849/02 $5.00 Copyright © 2002 the American Physiological Society
at basal levels; therefore, TSH is inappropriately low for the amount of circulating T4 (23). However, stimulation tests of the pituitary-thyroid axis by administration of exogenous thyrotropin-releasing hormone (TRH) reveal a normal rise in plasma TSH comparable to control levels, and a subsequent, appropriate rise in plasma free T4 and free T3 throughout the progressive course of hypothyroxinemia (23). This pattern is consistent with central hypothyroidism in humans and experimental animals (47, 52) and points to critical alterations of the thyroidal axis by central mechanisms leading to TRH deficiency.

TRH-containing neurons in the paraventricular nucleus (PVN) of the hypothalamus exert control over the biosynthesis and release of TSH from the pituitary (reviewed in Refs. 15, 31). Experimental hypothyroidism in rats results in a significant increase in prepro-TRH mRNA expression and peptide content in the PVN (35, 53, 57, 63) and hypothalamic TRH release (50). In the present study, we evaluated TRH transcript expression in the PVN by in situ hybridization in sleep-deprived and comparison rats. We also evaluated type II 5`-deiodinase (5`-DII) mRNA expression in the arcuate nucleus and median eminence (ARC-ME), known to be rich in 5`-DII and to show increased expression during thyroidectomy produced by chemicals or surgery (60). The principal finding is that prepro-TRH mRNA levels increase during prolonged sleep deprivation as an apparently appropriate response to decreased circulating thyroid hormone concentration, but expected TSH responses to peripheral hypothyroxinemia and to increased TRH transcript levels do not occur. 5`-DII mRNA expression in the ARC-ME was slightly but insignificantly increased in sleep-deprived animals rather than being suppressed, as would be expected if thyroid hormones were present in sufficient local excess to exert increased negative feedback on the PVN, and thereby contribute to downregulation of the thyroid axis. These findings point to posttranscriptional mechanisms and/or inhibitory factors as responsible for TRH deficiency and resultant hypothyroxinemia induced by sleep deprivation.

METHODS

Animals. All procedures were carried out in accordance with the National Institutes of Health guidelines on the care and use of animals and with an animal study protocol approved by the University of Tennessee Animal Care and Use Committee. Eighteen adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 464 ± 24 (±SD) g and aged 24 ± 2.5 wk were surgically prepared, of which six pairs served as sleep deprived and experimentally yoked animals, respectively, with six surgical controls. Anesthesia and analgesia were induced by ketamine·HCl (100 mg/kg ip), xylazine·HCl (2.4 mg/kg im), and atropine sulfate (0.1 mg/kg im). Supplementary doses of ketamine·HCl (10 mg/kg ip) were provided as needed. A solution of 1%–2% lidocaine·HCl (2.4 mg/kg) was also administered subcutaneously at an abdominal incision site through which a low-frequency telemetry transmitter (Barrows, San Jose, CA) was implanted to detect hypothermia, one indication of advanced morbidity (9, 21). Cortical and muscle electrodes were implanted for monitoring sleep and wakefulness. Animals were allowed to recover from surgery for ≥7 days.

Animal environmental conditions. Rats were kept under conditions of constant light to diminish the amplitude of the circadian rhythm. This was to avoid the influence of phase of circadian rhythm on dependent variables under study and the influence of sleep deprivation on the phase, which would differ between subject groups. Ambient air temperature was maintained at 28°C, within the thermoneutral zone established for rats (56), with thermostatically controlled heat lamps. Food and water were available ad libitum. Rats were fed a balanced, purified diet that was isoenergetic to a normal diet and augmented with protein, as used in previous studies (23, 27).

Procedure for producing sleep-deprived, yoked, and sham control animals. The apparatus and procedures for sleep deprivation are detailed elsewhere (9, 27). In brief, amplitude changes in electroencephalogram (EEG) variables were electronically processed to detect sleep onset in the rat to be sleep deprived, which triggered slow rotation of a platform on which the rats were housed, forcing both the sleep-deprived and the yoked rats to move for several seconds. The yoked rat experienced forced locomotion at the same time as the sleep-deprived rat, but it could sleep whenever the platform was stationary because the sleep-deprived partner was awake. This experimental paradigm results in consistent sleep loss across subject groups (21, 22, 24, 27). During a 7-day baseline period, with 6 s of rotation every hour, rats are awake 47% of the time. Thereafter, sleep-deprived rats are kept awake 90% of total time, and much of the remaining sleep is highly fragmented and/or consists of low-amplitude EEG transitional sleep. Yoked rats are awake 58% of total time, and their sleep is fragmented because of frequent arousals from sleep due to the paired conditions and are thus considered partially sleep deprived. Sham controls are permitted to sleep normally throughout the baseline and experimental periods.

Sleep-deprived rats exhibit a consistent progression of clinical signs, but individual animals do so at different rates (46). The only definitive clinical marker of severity of sleep deprivation is an eventual, acute terminal condition of advanced morbidity and hypothermia. We have used this retrospectively in other studies to determine the temporal course of each animal in proportion to its survival period (9, 22, 27). In the present study, sleep deprivation or control conditions were maintained for 15–21 days, which brackets the final quartile of survival defined in previous studies. We wished to produce sleep deprivation long enough for marked hypothyroxinemia and its sequelae to become manifest but short enough to preclude advanced morbidity and hypothermia. To these ends, food intake, body weight, and 24-h body temperature were monitored daily, as previously described (9, 27). Special food tubes with a waste receptacle afforded accurate measurement of food consumption (9). In four pairs of animals, the deprivation period extended to 20–21 days, by which time the fourth sleep-deprived rat had reached advanced morbidity. The subsequent two pairs of animals were studied after 16 days.

Tissue collection and processing. At the end of the experimental periods, rats were deeply anesthetized with injectable anesthetics and analgesics, and cardiac puncture was performed for exsanguination and blood sampling. The brain was removed and frozen in −40°C hexane. Blood that had been drawn into serum separator tubes during the cardiac puncture procedure was centrifuged, and the serum was stored at −80°C for assay of T4, T3, and TSH.
In situ hybridization. The prepro-TRH oligonucleotide (5'-GCTTTTTCCTCCTCTCCTTCTGTTGGATGCTG-GCTTTTGTGAT-3') was a 48-mer sequence used by other investigators (64) complementary to a region of the TRH transcript characterized in rat brain by Lechan et al. (39). The 5'-DII oligonucleotide (5'-GCTAATGGGTAGCCCTCATACGAATACGGC-3') was a 40-mer derived from the sequence published by Croteau et al. (13), complementary to a conserved region of Rattus norvegicus and Rattus rattus type II iodothyronine deiodinase that also has 90 and 88% homology with human and Rana catesbeiana sequences, respectively.

Frozen brain sections (16 μm) were cut on a cryostat at −18°C, collected on polylysine-coated slides, and processed for in situ hybridization as previously described (44). For each animal, a total of 10 sets of slides was collected, each set consisting of brain sections spaced at 160-μm intervals. In the present analyses, slides encompassing the extent of the hypothalamus were fixed in 4% paraformaldehyde in phosphate-buffered saline, acetylated, glycinated, dehydrated through ethanol, and extracted with chloroform. The sections were hybridized for 3 h at 37°C in a hybridization mix containing 20 pmol/ml 35S-labeled oligonucleotide probe in 2x SSC, 50% formamide, 10% dextran sulfate, 1x Denhardt’s solution, 0.5 mg/ml salmon DNA, 0.25 mg/ml yeast tRNA, and 100 mM dithiothreitol. After stringency washes in 2x SSC and 50% formamide at 42°C (prepro-TRH) or 37°C (5'-DII), sections were dehydrated through graded ethanol and exposed against Kodak SB-5 film for 10 days (TRH) or 7 days (5'-DII) together with 14C standards (Amersham Life Sciences, Arlington Heights, IL). Some films were also exposed for longer intervals to obtain 5'-DII images for illustration (for example, see Fig. 3A).

Brain sections from six rats per group were hybridized for prepro-TRH and 5'-DII mRNA, with the exception that five rats comprised the sham group for 5'-DII mRNA analysis. One yoked rat was excluded from quantitative analysis of prepro-TRH mRNA expression, because the sections that had been selected and hybridized with those of the other animals had not captured the appropriate level of hypothalamus. Another yoked rat was excluded from analysis of 5'-DII mRNA expression because the ARC-ME was damaged during dissection. The autoradiogram of each hybridized section was captured as a calibrated image by use of the NIH Image program, and those demonstrating peak hybridization signal at approximately the same rostral-caudal brain level were selected from each animal for quantitative densitometry of prepro-TRH mRNA in the PVN, the lateral hypothalamus (LH), and the reticular thalamic nucleus (Rt) and 5'-DII in the ARC-ME. For measurements of the PVN, Rt, and ARCM-E, the appropriate structure was thricel outlined within the border of greatest signal contrast from surrounding brain. For measurements of the LH, which exhibited scattered points of high signal density (see Fig. 2A), a standard size oval shape encompassing this structure was used to measure density within a fixed area across all sections. Bilateral measurements of PVN, Rt, and LH, minus background, were averaged to yield one value per structure for each animal. The investigators independently scanned and measured the prepro-TRH signal in the PVN, with good agreement (r = 0.96). Further evaluations of average hybridization derived from density measurements in fixed areas encompassing the PVN in two to three sequential sections yielded no change in relative signal intensity determined from peak sections, indicating that these provided representative sampling of PVN for each animal. The data presented and analyzed below were derived from PVN profiles in peak sections to better reflect the relative signal intensity in this nucleus vs. Rt and LH.

Hormone determinations. Serum from unoperated colony rats was assayed for quality control and to provide values for comparison with those of the treatment groups. Serum concentrations of total T4 and total T3 were determined in single-batch radioimmunoassays using commercial kits (Diagnostic Systems Laboratories, Webster, TX). The coefficient of variation with an assay was <3% for T4 and <6% for T3.

Serum TSH was determined by immunoassay by AniLytics (Gaithersburg, MD). The low and high sensitivities were 0.4 and >50 ng/ml, and the coefficient of variation with the assay was <4%.

Data analysis. A one-way analysis of variance (ANOVA) was used to determine a main effect due to treatment. Post hoc comparisons among the individual groups of sleep-deprived, yoked, and sham controls utilized the Bonferroni correction for multiple comparisons. For all analyses, statistical significance was considered when P < 0.05. Variance is given as means ± SD unless specifically noted as means ± SE.

RESULTS

Physiological variables and plasma concentrations of TSH, T4, and T3. Sleep-deprived rats were hyperphagic and lost weight. Daily food consumption averaged +205 ± 21% (range: +187 to +231%) of baseline amounts on each of the last 3 days of each experiment, and weight loss was −9.5 ± 4.8% (range: −10.0 to −15.3%) of baseline at the time of tissue collection. Corresponding food consumption and body weight changes in yoked controls were +148 ± 25% (range: +114 to +174%) and +3.8 ± 1.5% (range: +1.6 to +5.3%), whereas respective values in sham control rats were +109 ± 10% (range: +100 to +125%) and +5.0 ± 4.0% (range: +1.7 to +10.7%). Core temperature was assessed for a decline >1°C below baseline during 24 h, indicative of advanced morbidity, which occurred in one rat sleep deprived for 21 days.

Total T4 differed significantly among all treatment groups (F2,15 = 41.2; P < 0.0001), being most profoundly reduced in the sleep-deprived group (sleep deprived vs. yoked, t = −5.97, P < 0.02; sleep-deprived vs. sham, t = −7.81; P < 0.02), with an intermediate reduction in the partially sleep-deprived, yoked group (yoked vs. sham, t = −4.42 P < 0.02) (Fig. 1). Total T3 concentration did not differ among groups (sleep deprived, 1.6 ± 0.2; yoked, 1.5 ± 0.2; sham, 1.6 ± 0.1 nmol/l). Despite marked differences in T4 concentration, TSH concentrations did not significantly differ among groups (sleep deprived, 2.8 ± 1.5; yoked, 1.9 ± 0.9; sham, 1.7 ± 0.3 ng/ml). The T4 and TSH results for individual sleep-deprived, yoked, and sham treatment animals are shown in Fig. 1.

Prepro-TRH and 5'-DII mRNA expression in the sleep-deprived brain. Representative autoradiographic images in Fig. 2 illustrate the distribution of TRH transcripts in sham control and sleep-deprived rat brain, showing strong expression in the hypophysiotropic area of the PVN, as well as in the LH and Rt. Quantitative hybridization results demonstrate that prepro-TRH mRNA expression in the Rt and LH did
not differ among sleep-deprived, yoked, and sham control groups. Prepro-TRH mRNA expression in the PVN showed a significant increase in the sleep-deprived group vs. sham control \( (P < 0.02) \) and a similar trend that did not reach significance in the yoked group.

Representative autoradiographic images of 5'-DI1 mRNA expression in the ARC-ME region are shown in Fig. 3 together with quantitative hybridization results for the three experimental groups. Although mean 5'-DI1 hybridization tended to be higher in sleep-deprived animals (sleep deprived: 99 ± 24; yoked: 76 ± 11; sham control: 82 ± 19 nCi/g), there were no significant differences among the groups. Post hoc review of 5'-DI1 mRNA data showed that two sleep-deprived animals displayed a signal >2 SD above the average of the sham control group, and in all cases individual values were greater than the average of the sham control group.

Post hoc evaluation of results for individual animals as a function of the duration of sleep deprivation showed that neither of the sleep-deprived rats studied at 15–16 days showed elevated TRH transcript levels in PVN, whereas three of the four animals studied at 20–21 days averaged approximately three times the control hybridization signal (Fig. 4). The one sleep-deprived animal that did not exhibit elevated prepro-TRH mRNA at 21 days was also the only rat exhibiting advanced morbidity in this study, characterized by a 24-h drop in core temperature of >1°C. The two highest values for prepro-TRH mRNA expression in yoked...
animals were also observed at 20–21 days. These results demonstrate that upregulation of TRH transcription is a late event in the course of sleep deprivation.

Post hoc evaluation of the relationships between hormone concentration and transcript levels within groups did not reveal statistically significant correlations between T₄ concentrations and either TSH concentrations or prepro-TRH transcript levels, nor between TSH concentrations and TRH transcript levels, owing in part to low variability in T₄ and TSH concentrations. However, sleep-deprived animals studied after 20–21 days tended to have higher TSH concentrations than animals studied after 15–16 days (Fig. 4).

DISCUSSION

Sleep deprivation resulted in the characteristic spectrum of changes observed in previous studies, including weight loss and dramatically increased food consumption (22, 27) and marked hypothyroxinemia (8, 23) (Fig. 1). The progressive decline in circulating total and free T₄ and T₃ observed in previous studies indicates that the mechanisms responsible for decreased hormone production/release are affected early and persistently. Previous investigations suggested that alterations in central mechanisms regulating TRH synthesis and release may be responsible, since sleep-deprived rats with low thyroid hormone levels and unchanged TSH respond to exogenous TRH administration with an appropriate burst of TSH release and thyroid activation throughout the average 21-day experimental period (23). The stimulated TSH release in sleep-deprived rats is not blunted, which, when it occurs in response to food restriction (49), may be considered part of an adaptive response to lower energy requirements. The thyroid hormone profile of the sleep-deprived rat is consistent with central hypothyroidism in humans (47, 52) and resembles TRH deficiency in rats produced by lesions of the PVN, which results in

![Fig. 3] 5'-Deiodinase type II (5'-DII) mRNA expression in hypothalamus and the effect of sleep deprivation. Left: representative autoradiograms illustrate in situ hybridization of 5'-DII mRNA in the arcuate nucleus-medial eminence (ARC-ME) and periventricular region (PE) of a sham control animal (top) and a sleep-deprived animal (bottom). The films were subjected to prolonged exposure relative to those used for densitometry to visualize more clearly the structures expressing 5'-DII mRNA. Illustrated fields are ~4.5 mm in width. Right: quantitative hybridization results in sleep-deprived, yoked, and sham control groups. There were no statistically significant group differences. Error bars, SE.

![Fig. 4] Time course of prepro-TRH mRNA and TSH responses during sleep deprivation. Results from individual animals are illustrated for prepro-TRH mRNA expression in the PVN (A) and for serum TSH concentrations (B). Yoked (○) and sleep-deprived (■) animals were studied after 15–16 or 20–21 experimental days, when there was marked hypothyroxinemia in the sleep-deprived animals. The blocked area represents the mean ± SD of control values, derived from individual animals indicated at day 0 (●).
decreased plasma T₄ and T₃ (43), decreased plasma TSH response to hypothyroidism (3, 43), but normal TSH secretion in response to exogenous TRH administration (3).

Prepro-TRH mRNA expression in the PVN showed a significant increase in the sleep-deprived group, and a similar trend in the yoked group, relative to the sham control group. TRH transcript levels in the LH and the Rt did not differ among groups (Figs. 2 and 4), consistent with preferential upregulation of TRH biosynthesis in the hypophysiotropic pathway (19, 35, 53, 57). Post hoc evaluation of individual sleep-deprived animals revealed threefold elevations in three of four animals studied at 20–21 days but not in those studied after shorter duration despite nearly comparable hypothyroxinemia. Many days also are required to observe increased prepro-TRH mRNA in experimental models of thyroid hormone depletion. T₄ is diminished after chemical or surgical thyroidectomy to a concentration much lower than that observed in sleep-deprived rats within 3–6 days (29, 30, 38) without an appreciable increase in TRH transcript levels in the PVN (38). Thereafter, prepro-TRH mRNA begins to rise (38), concurrent with TRH depletion in the median eminence and much of the hypothalamus (63). In thyroidectomized animals, prepro-TRH mRNA levels reach 155–190% of the euthyroid level by 14 days (38, 42, 63) and 200–250% of euthyroid control by 21 days or longer (11, 12, 34, 38, 53). These peak changes are comparable to those observed in this study, and the lack of accumulation of TRH transcripts in 16-day sleep-deprived animals may be consistent with the more gradual progression to a severely hypothyroxinemic state during sleep deprivation (8, 23) than during experimental hypothyroidism. However, yoked animals tended to show elevated prepro-TRH mRNA expression at 20–21 days despite more moderate decreases in peripheral T₄ (Fig. 4) (8, 23), indicating that the duration of hypothyroxinemia may be as important a factor as its severity in triggering this response. TRH transcripts were not elevated in one sleep-deprived rat studied after 21 days, but this was accompanied by advanced morbidity. It remains to be determined whether failure to upregulate TRH expression may result in a more severe pathological course or, conversely, whether an abrupt decline in prepro-TRH mRNA expression may be associated with the terminal phase of sleep deprivation.

A major feature distinguishing thyroidectomy and sleep deprivation is the TSH response. Sleep-deprived animals in the present study showed no change, or only slight increases, in plasma TSH at the end of the deprivation period. This is consistent with a previous study that utilized indwelling catheters for repeated blood sampling that revealed unchanged TSH across experimental days despite progressive hypothyroxinemia (23). In contrast, TSH levels rise during chemical thyroidectomy even before serum T₄ or T₃ shows a detectable change (40). Modest declines of serum T₃ by 11–24% during 4 days of low-dose methylmercaptoimidazole or propylthiouracil treatment, with little or no change in serum T₃, are associated with three- to fourfold increases in serum TSH (40). Several-fold increases in TSH occur by 4–6 days after thyroidectomy treatments (29, 30, 38), before any detectable change in prepro-TRH mRNA expression (38). As experimental hypothyroidism is prolonged beyond this initial period, TSH levels increase progressively to more than 10-fold euthyroid levels (29, 30, 38). Maintenance of 50% of normal plasma T₄ in thyroidectomized animals by supplementary T₄ administration for 14 days is associated with an eight- to ninefold elevation of plasma TSH concentration (16). The blunted TSH response in sleep-deprived animals, therefore, is inappropriate, given both the magnitude and duration of hypothyroxinemia and the above-demonstrated increase in TRH transcripts.

TRH deficiency produced by PVN lesions likewise results in a markedly blunted TSH response to hypothyroidism. Serum TSH concentrations in PVN-lesioned animals show only 30% of the increase seen in thyroidectomized rats with intact PVN (30, 57). This is associated with a dramatically increased TSH in TSH subunit mRNA levels in anterior pituitary in response to hypothyroidism of 2–4 wk duration (57). The threshold for thyroid hormone feedback onto the pituitary adenohypophysis is lowered in PVN-lesioned or deafferented animals (29, 41), such that even small doses of T₄ are effective in completely suppressing the marginal increases in serum TSH (41). On the basis of the similarities between sleep-deprived and PVN-lesioned animals, TRH deficiency remains a more compelling explanation for thyroid axis suppression than does primary TSH deficiency, particularly in light of the fact that sleep-deprived rats respond to exogenous TRH administration with an appropriate increase in serum TSH (23). The present results, demonstrating appropriate increases in TRH transcript levels in PVN, direct attention to factors regulating the translation of prepro-TRH mRNA, processing and release of TRH peptide, and/or feedback responsiveness of pituitary thyrotrophs as the foci of altered thyroid hormone regulation in sleep-deprived animals.

An additional comparison between PVN-lesioned and sleep-deprived rats concerns the T₄-to-T₃ conversion enzyme 5'-DII. This enzyme is upregulated in response to decreased circulating T₄, with identical concentration dependence of activities in pituitary, cerebral cortex, and BAT (54, 55). Prolonged experimental hypothyroidism increases pituitary 5'-DII activity to 400–900% of euthyroid levels (13, 48). This response is not reversed by subsequent PVN lesions, but such lesions in euthyroid animals reduce plasma T₄ and T₃ by 60 and 75%, respectively, yet produce only a moderate 50% elevation of pituitary 5'-DII at 1 wk (43). A previous study of rats sleep deprived for 14 days likewise revealed only a modest, statistically insignificant 40% increase in 5'-DII activity in the pituitary, whereas that in BAT was increased 100-fold (6). Supplementation T₄ treatment partially reduced the BAT 5'-DII activation during sleep deprivation (6), indicating a sensitivity to T₄ that would predict a larger than
observed increase in pituitary 5'-DII activity. The attenuated responsiveness of pituitary 5'-DII activity may be indicative of comparable TRH deficiency in PVN-lesioned and sleep-deprived animals.

Regulation of 5'-DII in responses to changing thyroid hormone status has also been investigated in brain. Studies in cerebral cortex indicate that, although enzyme activity is primarily responsive to T4, 5'-DII mRNA expression is inversely related to T3 levels (10). Local production of T3 from T4 via 5'-DII in astrocytes (17) and tanyocytes (60) of the ARC-ME has been proposed to play a specific role in signaling the PVN, which lacks 5'-DII (48). We found moderate increases in 5'-DII mRNA expression in ARC-ME of two sleep-deprived animals (Fig. 4), consistent with the modest 30–60% elevations observed after hypothyroxinemia resulting from fasting (18) or thyroidectomy (16, 60). The greater difficulty in achieving consistent sample locations in sections through the ARC-ME renders the quantitation of 5'-DII mRNA expression somewhat less reliable than the prepro-TRH measurements. Nevertheless, there was clearly no indication of reduced 5'-DII mRNA in the ARC-ME that might indicate local T3 excess. In comparison, 5'-DII mRNA levels in ARC-ME are decreased in euthyroid animals treated with T4 (18). Feedback action of thyroid hormones exerted at the level of the hypothalamus is considered an important factor affecting TRH release (51) as well as its production. Although we did not specifically evaluate 5'-DII mRNA levels in the periventricular area (PE), there was likewise no indication of the severalfold increase observed in this region that appears uniquely induced by fasting and associated with enhanced feedback downregulation of prepro-TRH expression in the PVN by T3 (18) (see Fig. 3).

Recent reports have described acute upregulation of 5'-DII activity in several brain regions in response to diverse stresses, including a model of forced walking with an eventual period of short-term sleep deprivation (7, 20). In the latter paradigm, increased activity was particularly prominent in frontal cortex. Because enzyme activity and mRNA levels can be regulated independently (see above), it is difficult to relate such observations to the present hybridization results. The levels of 5'-DII mRNA are much lower in cortex than ARC-ME, and we saw no suggestion of a change in transcript levels in cortex. It should be noted that reported increases in enzyme activity were more prominent after 10 h of forced mobility than after the longer intervals that included sleep deprivation (7), further indicating that this is an acute response unlikely to be relevant to the model of prolonged sleep deprivation employed in the present study.

Besides thyroid hormones, several nonthyroidal factors affect TRH translation, production, and release via short and long control loops and therefore have the potential to explain thyroid axis suppression during sleep deprivation. Hypothetically, sustained wakefulness may lead to decreased neuronal stimulation of the PVN by other brain regions. Electrical stimulation, used to mimic neuronal excitation of the PVN, results in increased TRH release through the portal system, resulting in elevated plasma TSH (15). Under certain conditions, such as exposure to cold, ascending noradrenergic and/or adrenergic projections to the PVN (reviewed in Refs. 38, 64) appear to override the normal inhibitory effects of increased T3 and produce increased TRH mRNA expression in the PVN (64). To assess functional activation in the sleep-deprived rat brain, a study of metabolic activity revealed that glucose metabolism is decreased in most hypothalamic structures except the lateral hypothalamic feeding “on” center (24). Although the PVN had not been explicitly quantitated in that study, the particularly low metabolic rates of adjacent structures indicate a decreased metabolic activity that may reflect tonic inhibition in this region. Another candidate factor, dopamine, exerts inhibitory control on TRH at two central levels, affecting both hypothalamic TRH release (4) and pituitary TSH responses to TRH (37). L-Dopa administration to hypothyroid patients lowers elevated TSH levels without changing the response to TRH (reviewed in Ref. 37). Abundant evidence shows that dopamine acts on pituitary thyrotrophs (reviewed in Ref. 37). Recent evidence has also linked dopamine to the regulation of the expression of 5'-DII mRNA in the ARC-ME via a dopamine- and cAMP-regulated phosphoprotein found in tanyocytes (28), which may be relevant insofar as 5'-DII mRNA was not found to be robustly increased in this region in the present study.

Several additional nonthyroidal factors are implicated in sleep deprivation-induced hypothyroxinemia by virtue of coexisting metabolic (8, 24, 27) and host defense abnormalities (21, 26). Neuropeptide Y can induce food-seeking behavior and hyperphagia (58), which are prominent features of the sleep deprivation profile. Neuropeptide Y is contained in neurons innervating TRH cells (59) and appears to block catecholaminergic inputs to the PVN (reviewed in Ref. 38). Endotoxin and cytokine exposure, implicated in the hypercatabolic and immune-suppressed state of the sleep-deprived animal (26), produces key characteristics of central hypothyroidism, including low plasma T4 and T3 but inappropriately normal or decreased TSH levels (33, 36, 62). Conversely, thyroid axis suppression by glucocorticoids is not a candidate mechanism, because overactivation of the hypothalamo-hippocampal-adrenal axis has not been found in this model (23), and the present results provide direct evidence against glucocorticoid-induced reductions in prepro-TRH mRNA expression in PVN (32).

In summary, sleep deprivation produces the paradoxical combination of peripheral hypercatabolism and central hypothyroxinemia. Other clinical states to which sleep deprivation may be typically compared are associated with suppression of TRH mRNA expression in the PVN, which appears to explain suppression of the thyroid axis in these cases. In contrast, increased prepro-TRH mRNA expression was demonstrated in the sleep-deprived brain, consistent with compensatory TRH biosynthesis. These data suggest that the locus of abnormal thyroid hormone regulation lies after...
prepro-TRH transcription, at steps up to and including release of the mature peptide. Features of the sleep-deprived profile point to nonthyroidal control mechanisms, such as inhibitory neurotransmitters and neuromodulators, including those related to immunity, as potential candidates mediating central hypothyroxinemia induced by sleep deprivation.

We thank Dr. Qihong Zhou for preparation of frozen sections, and Jason M. Scott for technical assistance.

Research support was provided by the National Institute of Neurological Disorders and Stroke (NS-38733) and the National Heart, Lung, and Blood Institute (HL-59271).

REFERENCES


29. Greer MA, Sato N, Wang X, Greer SE, and McAdams S. Evidence that the major physiological role of TRH in the hypothalamic–paraventricular nuclei may be to regulate the set-point for thyroid hormone negative feedback on the pituitary thyrotroph. Neuroendocrinology 57: 569–575, 1993.


35. Kondo K, Harbuz MS, Levy A, and Lightman SL. Inhibition of the hypothalamic-pituitary-thyroid axis in response to lipo-
polysaccharide is independent of changes in circulating cortico-


40. **Mannisto PT, Ranta T, and Leppaluoto J.** Effects of methyldopaminediazole (MDM), propylthiouracil (PTU), potassium perchlorate (KClO4) and potassium iodide (KI) on the serum concentrations of thyrotrophin (TSH) and thyroid hormones in the rat. *Acta Endocrinol (Copenhen) 91: 271–261, 1979.


56. **Szymusiak R and Satinoff E.** Maximal REM sleep time defines a narrower thermoneural zone than does minimal metabolic rate. *Physiol Behav* 26: 587–590, 1981.


64. **Zoeller RT, Kabeer N, and Albers HE.** Cold exposure elevates cellular levels of messenger ribonucleic acid encoding thyrotropin-releasing hormone in paraventricular nucleus despite elevated levels of thyroid hormones. *Endocrinology* 127: 2955–2962, 1990.